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Beth A. Beidleman, Janet E. Staab, Stephen R. Muza, Michael N. Sawka

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USARIEM TECHNICAL REPORT T15-1

PREDICTED HEMATOLOGIC AND PLASMA VOLUME RESPONSES FOLLOWING RAPID ASCENT TO PROGRESSIVE ALTITUDES

Beth A. Beidleman
Janet E. Staab
Stephen R. Muza
Michael N. Sawka

Thermal and Mountain Medicine Division

June 2014

U.S. Army Research Institute of Environmental Medicine
Natick, MA 01760-5007

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Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and the USAMRMC Regulation 70-25 on the use of volunteers in research

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ACKNOWLEDGEMENTS

The dedicated and professional efforts of Dr. Charles Fulco, Dr. Allen Cymerman, Dr. Paul Rock, Ms. Claudia Toussaint, Mr. Timothy Driscoll, and Mr. Sean Andrew in conducting the altitude studies, maintaining the database, and collating the data supporting the development of these predictive models are acknowledged and greatly appreciated. The dedication and efforts of the test volunteers in completing these altitude studies are also acknowledged and appreciated.

EXECUTIVE SUMMARY

Scientific consensus does not exist regarding hematological responses to moderate to high altitude exposure. The US Anti-Doping Agency (USADA) requested the US Army Research Institute of Environmental Medicine (USARIEM), to predict human hematologic and plasma volume (PV) responses of lowlanders to rapid ascent and stay at moderate to high-altitudes. The purpose was to quantify the expected changes in hemoglobin concentration [Hb], hematocrit (Hct), and PV change as a result of a given gain in terrestrial elevation, delineate the time course of these changes, and define baseline demographics and physiologic descriptors that are important in predicting these changes. Using general linear mixed models and a comprehensive relational database containing individual ascent profiles, we analyzed 999 values of [Hb], Hct and PV change following rapid ascent (< 6 h) in 361 male and female unacclimatized lowlanders who spent between 2 hours and 16 days at 1850 m to 4501 m under well controlled experimental conditions. Covariates included in the analysis were altitude, time at altitude, quadratic effect of time at altitude, initial [Hb] and Hct status, gender, age, body mass index, race, smoking status, and aerobic fitness level (i.e., sea-level VO_{2max}). The [Hb] and Hct increased over time at altitude ($P<0.0001$) due to rapid PV loss and the response was impacted by altitude ($P<0.0001$), gender ($P<0.05$), and initial values of [Hb] and Hct ($P<0.0001$). The overall impact of altitude can be summarized using the following correction factors after one day at altitude: 1) 0.1 g/dl increase in [Hb] for every 500 m increase in altitude regardless of gender, 2)1.1% increase in Hct at 2000 m with an additional 2-6% increase for every 500 m increase in altitude depending on the altitude, and 3) rapid 5% loss in PV at 2000 m and additional 0.6% decrease with every 500 m above 2000 m. Individuals with a low initial [Hb] and Hct demonstrated a more dramatic increase over time at altitude than those with a high initial [Hb] and Hct. Aerobic fitness level was not a significant predictor in the model. In conclusion, hematological responses to altitude are dependent upon the exposure elevation and duration of exposure with small but significant effects of gender and initial values. It is anticipated that results will enhance the ability of sport organizations to detect "blood doping" in athletes seeking to gain an edge in their athletic performance.

INTRODUCTION

Blood doping in sport is an ethical concern and research is increasingly being directed at catching "cheaters" [1]. Erythrocyte volume expansion can markedly improve aerobic performance and tolerance to environmental extremes [2,3] and such manipulation often results in acute plasma volume (PV) loss and elevated hemoglobin concentrations [3]. Detection of unethical and illegal manipulation of erythrocyte volume by following changes in hemoglobin concentration ([Hb]) in elite athletes is a problem for agencies that monitor "fair play" in sport [4,5]. "Live-high and train-low" has been shown to be an effective method to legally increase erythrocyte volume in some athletes [6] and athletes, therefore, frequently report some prior altitude exposure. Altitude exposure will increase [Hb] most often due to PV loss [7] and these changes are likely related to the magnitude and duration of altitude exposure. Prolonged altitude exposure also induces erythrocyte volume expansion which results in further increases in [Hb] [8]; however such relationships regarding exposure duration and magnitude are based on studies employing vastly different methodologies, populations and altitude exposures.

Sport anti-doping groups have applied an "altitude correction factor" when athletes can document recent altitude exposure, but it remains unknown whether the magnitude of this correction factor is accurate [9]. In addition, the expected time course of changes in [Hb], Hct and PV at various altitudes has never been defined while controlling for demographic factors such as age, body mass index (BMI), aerobic fitness level, race, gender, smoking status, and initial hematologic status in a comprehensive data set. The purpose of this study was to develop models to predict hematologic and PV responses following rapid ascent to various altitudes in unacclimatized lowlanders and delineate the time course of these responses over the first 7days of exposure. This was accomplished using a comprehensive relational database containing individual ascent profiles, demographics, and physiologic subject descriptors as well as repeated measures of [Hb] and Hct from 19 carefully conducted and well-controlled experimental studies employing similar methodologies.

LITERATURE REVIEW

Numerous research studies have examined the hematologic response in unacclimatized lowlanders ascending to a given altitude [6,10-29]. Interpretation of prior studies is extremely difficult due to marked differences in subject populations, experimental procedures and conditions. These studies have looked at the acute (< 3 weeks), chronic (3-6 weeks), and long-term (8 mo-1 year) hematologic response of various cohorts following exposure to altitudes ranging from 1800 m to 5500 m. In general, these studies demonstrate that [Hb] and Hct increase rapidly in lowlanders following an acute exposure to altitude due to an abrupt reduction in PV [7]. With chronic exposure to altitudes around 4000 m, erythrocyte volume expansion occurs due to an erythropoietin-stimulated increase in red blood cell production [22,28]. With long-term exposure to altitude, PV recovers and erythrocyte volume continues to expand so that total blood volume is increased [8,24]. Native highlanders consistently demonstrate larger total blood volumes than sea-level residents [13,30-32]. Upon return to sea level (SL), PV quickly recovers (< 1 week) to pre-ascent values in acclimatized lowlanders [14,16,19] whereas native highlanders can take up to 4 months to return to normal values [13] (See Table 1).

One extensive review article [7] and one meta-analysis [8] summarized the results of many studies that have examined the hematologic and PV response to various altitudes. During an acute altitude exposure, PV reductions are greater at higher compared with lower elevations [7]. In addition, the PV response at altitude is time-dependent [8]. The initial decrease in PV is followed by a more pronounced decrease with chronic exposure [6,13,27] that gradually return towards normal sea-level values following 4-8 months of altitude exposure [6,13,27]. However, [Hb] and Hct remain elevated due to stimulation of erythrocyte production. Erythrocyte production is accelerated at higher altitudes with expansion taking two weeks at altitudes greater than 4000 m but four weeks at altitudes less than 3000 m [8]. In addition, the erythropoietic response may be dependent on the initial erythrocyte volume. Individuals with a high initial erythrocyte volume may demonstrate less of an increase during an altitude sojourn compared to those with a low initial erythrocyte volume [8].

Table 1. Summary of Studies Measuring Hemoglobin [Hb], Hematocrit (Hct), and Plasma Volume (PV) responses During a Period of Return to Sea Level Following Altitude Acclimatization

Study	Population	Gender	Altitude (m)	Exposure Duration (days)	Activity During Exposure	Return to SL Exposure (days)	Hematologic Response from Pre-Exposure Values
Svedenhag et al. (1997) [33]	Elite endurance athletes	Men and Women (n=7)	1900	30	Vigorous Training	1, 11 and 35	NS change in [Hb] days 1, 11, or 35
Faulkner et al. (1967) [34]	College swimmers	Men (n=21)	2300	14-21	Vigorous Training	3 and 21	NS change in [Hb] or Hct days 3 or 21
Dill et. al (1974) [35]	Elite athletes	Men (n=12)	2300	21	Vigorous Training	1, 2, 4, 8, 12, 16 and 20	P<0.05 increase in Hct on days 1 & 4 NS change in Hct days 8,12,16 & 20
Gunga et al. (1996) [36]	Untrained Lowlanders	Men (n=5)	3600	8	Working in mines	1 and 3	NS change in [Hb] and Hct on days 1 & 3
He et al. (2013) [32]	Untrained Lowlanders	Men (n=87)	3650	180	Working in factory	3, 50, and 100	NS change in [Hb] and Hct on days 3, 50 & 100
Dill et al. (1969) [14]	Untrained Lowlanders	Men (n=12)	3800	14-40	Limited activity	1	NS change in [Hb] or PV on day 1
Krzywicki et al. (1969) [19]	Untrained lowlanders	Men (n=15)	4300	12	Vigorous Training	4 and 7	NS change in PV at days 4 & 7
Hannon et al. (1969) [16]	Untrained Lowlanders	Women (n=8)	4300	65	Limited activity	14	NS change in [Hb], Hct or PV on day 14
Lyons et al. (1995) [15]	Trained Lowlanders	Men (n=6)	4300	14	Limited Activity	1 and 6	P<0.05 increase in [Hb], Hct and PV on days 1 & 6
Reynafarje et al. (1959) [13]	Untrained lowlanders	Men (n=4)	4550	180	No training	7-10	P<0.05 increase in [Hb] on days 7-10
Grassi et al. (1996) [17]	Untrained Lowlanders	Men (n=10)	5050	35	Limited activity	7	NS change in [Hb] and Hct on day 7
Singh et al. (2003) [18]	Untrained Lowlanders	Men (n=15)	3500-5800	130	Limited Activity	7	NS change in [Hb] and Hct on day 7
Pace et al. (1956) [37]	Mountain Climbers	Men (n=10)	3360-7160	77	Climbing	17	P<0.05 increase in [Hb] on day 17
Savourey et al. (1996) [38]	Mountain Climbers	Men (n=9)	5000-8846	41-62	Climbing	10, 30 and 60	P<0.05 increase in [Hb] and Hct days 10 & 30 NS change in [Hb] and Hct day 60
Robach et al. (2000) [39]	Mountain Climbers	Men (n=8)	4300-9000	38	Climbing	1-3	P<0.05 increase in [Hb] and Hct on days 1-3

*NS=Non-significant; P<0.05=Significant

METHODS

Research Studies Employing an environmentally controlled hypobaric chamber and a laboratory on the summit of Pikes Peak, the US Army Research Institute of Environmental Medicine (USARIEM) has been able to collect blood data (999 data points) on 361 unacclimatized (no altitude exposure in the previous 3 months) men (n=256) and women (n=105) after rapid ascent (< 6 h) and stay at fixed altitudes (200-4500 m) under controlled conditions (no medication use, adequate hydration, physical activity assessment, controlled temperature and humidity) and enter this information into a relational database.

Eight studies were conducted in natural altitude conditions and 11 were conducted in the USARIEM hypobaric chamber. No difference in mean [Hb], Hct, and PV responses were detected between subjects participating in natural and hypobaric environment experiments. Ascent times in the hypobaric chamber were more rapid (< 15 min) than ascent times in the mountains (< 6 h). However, the time variable did not start until arrival at the destination altitude. Volunteers did not participate in physical training during prolonged stays at altitude but participated in exercise tests. In studies that used medication, only placebo volunteers were included. Recruitment procedures were the same (i.e, both sexes included and age range limited to 18- to 45- yr-olds) except one study conducted on men only (Special Forces unit) and two studies conducted on women only by design. None of the volunteers participated repeatedly in any of the studies.

Table 2 contains the mean, standard deviation (SD) and range of variables from the subject population used to develop the predictive models. Given that sea-level peak oxygen uptake (VO_{2peak}) was assessed in 271 of the 361 volunteers, a separate analysis was conducted with and without VO_{2peak} as a covariate. If a volunteer's sea-level VO_{2peak} was ≤ 50 ml/kg/min then they were assigned a fitness level of low. Women represented approximately 29% of the data set with an equal distribution across altitudes (15-25%) and both men and women fell into the same age quartiles (18-20, 21-23, 24-27, and 28-39). The mean exposure altitude was 3750 m with 63.9% of the individuals exposed to altitudes > 3750 m. A limited number of subjects in our data set (8%) were exposed to altitudes for > 7 days. Conclusions from the model are limited beyond this time frame.

All volunteers were healthy, well nourished, and physically active. All received

medical examinations and none had any preexisting medical conditions that warranted exclusion from participation. Each gave written and verbal acknowledgement of their informed consent and was made aware of their right to withdraw without prejudice at any time. The studies were approved by the appropriate Institutional Review Boards. Investigators adhered to the policies for protection of human subjects as prescribed in Army Regulation 70-25, and the research was conducted in adherence with the provisions of 32 CFR Part 219

Table 2. Characteristics of Unacclimatized Men (256) and Women (n=105) Lowlanders (n=361; 999 data points) utilized in the data set.

VARIABLE	MEAN	SD	MIN	MAX
Age (yr)	24.1	4.9	18	42
Weight (kg)	74.5	13.0	46.4	114.6
Height (m)	1.74	0.09	1.47	1.97
Body-mass index (kg/m^2)	24.6	3.1	18.4	35.1
Altitude (km)	3.75	0.68	1.92	4.50
Men (%)	70.9			
Age ≤ 30 years (%)	84.6			
Maximal oxygen uptake (ml/kg/min) (n=271)	48.9	7.4	30.0	72.9
Fit ≥50 ml/kg/min (%) (n=271)	43.2			
Smokers (%) (n=295)	15.6			
White Caucasians (%)	88.1			
Sea Level [Hb] (g/dl)	14.2	1.3	10.5	18.2
Sea-Level Hct (%)	42.2	3.7	31.0	53.5
Number of Measurements	2.8	0.7	2	6
Exposure Duration ≤ 8 days (%)	91.2			

[Hb], hemoglobin concentration, Hct, hematocrit, SD, standard deviation, MIN, minimum, MAX, maximum.

Dependent variables. The [Hb] and Hct were assessed at various time points depending on the protocol for each study. In addition to a sea-level baseline measurement of [Hb] and Hct, a minimum of one measurement and maximum of five measurements were made per individual over the first 1- to 16-days of exposure to various altitudes to delineate the time course of changes in hematologic and PV status.

In all protocols, venous measures of [Hb] and Hct were determined using an automated blood analyzer (i-STAT by Abbott Diagnostics or OSM3 Hemoximeter by Radiometer) and the Hct was either centrifuged and read manually or calculated from automated measures of [Hb]. Although Hct can vary using the two different methods of measurement when volunteers are dehydrated [40], all of our volunteers were euhydrated. The change in PV was calculated using the Dill and Costill equation [41].

Independent variables. Altitude coded in kilometers (i.e, one unit increase in altitude was equivalent to a 1000-m increase in altitude), time coded in 24-h increments (one unit increase in time was equivalent to 24 h), gender (men=0 and women=1), sea-level aerobic fitness level (low=0 and high=1) and initial sea-level [Hb] and Hct status as well as interactions were entered as major predictor variables in the model based on exploratory data analysis The following covariates were also included in the model: age, body mass index (BMI) (weight/height^2), race (white=0 and all others=1), and smoking status (> 3 months non-smoker=0 and smoker=1).

Statistical analyses. Five steps are typically followed when modelling longitudinal data. First, in order to determine the appropriate shape of the individual growth model associated with the hematologic response over time at altitude (i.e, linear, quadratic, exponential),all data are plotted and a smoothing line is created without considering the effect of independent variables on the dependent variable. Second, exploratory data analysis considers the relationships between certain independent (i.e., altitude) and dependent variables of interest (i.e, PV). Third, model development and interpretation proceeds by systematically eliminating non-significant variables from the model and determining the most parsimonious model that explains the largest amount of variability. Fourth, model assumptions are evaluated (i.e, normality, collinearity, residual analysis, outlier detection). Fifth, the model is internally validated using Efron bootstrap sampling or equivalent method [42].

We modeled [Hb], Hct, and PV responses over time at altitude using individual growth models containing subject-specific intercepts and slopes using PROC Mixed (SAS 9.2, Cary, NC) [43,44]. General linear mixed models allow the intercepts and slopes to vary by individuals such that individual predictions of changes in [Hb], Hct, and PV can be calculated for each subject in the data set. An individual growth model for pattern of change was assessed by regressing time, time^2, and time^3 on [Hb], Hct, and PV as both fixed and random effects. If higher orders of time were not significant ($P<0.05$), they were dropped from the model as both fixed and random effects and the

model was rerun. If predictor variables are not centered on their mean, the intercept represents the prediction for [Hb], Hct and PV when all predictors in the equation are zero (i.e., altitude=0 m, time=0 days, gender=0 or male and initial [Hb] and Hct=0). Given that zero is not a valid initial [Hb] and Hct value, both [Hb] and Hct were centered at their respective means (14.2 g/dl and 42.2%, respectively). After determining a suitable parsimonious individual growth model, all level 2 covariates and their interactions with time and each other were included in the model. Nonsignificant covariates ($P>0.10$) and their interactions with time and each other were eliminated from the model one at a time starting with the least significant effect until the final model was determined. A pseudo R^2 statistic was calculated by computing the predicted outcome value for each person on each occasion of measurement and squaring the the sample correlation between observed and predicted values [44]. The pseudo R^2 statistic was used to assess the total outcome variability explained by the predictors. Statistical significance was set at $P< 0.05$.

Model diagnostics for general linear mixed models were performed to compare the data with the fitted models to highlight any discrepancies. Diagnostic tools included residual analysis, outlier detection, influence analysis, and model assumption verification. There were no systematic trends in the residuals that indicated a mis-specified model. Seventeen to nineteen subjects had either a [Hb], Hct or PV response that were potential outliers but after careful inspection, it was determined the data were not erroneous. The distribution of the random effects for intercept, time, and time^2 were all normally distributed assessed by skewness and kurtosis statistics. Internal validation of the three models was conducted using Efron bootstrap resampling with replacement on 1000 bootstrap samples [42]. The difference between the root mean square error for the three models and root mean square error for the 1000 bootstrap samples was small and within the measurement error of the blood autoanalyzers.

RESULTS

Figures 1-2 depict two graphs from our exploratory data analyses. These figures do not represent regression equations but depict average trend lines to demonstrate how the initial growth model and variables of interest (i.e., altitude) are selected to include in the model. Figure 1 demonstrates a large initial drop in PV that levels off which suggests including a quadratic effect of time in the model. Figure 2

demonstrates that altitude is a likely candidate for inclusion in the PV model. Although both gender and fitness level were likely candidates to include in the model, the two variables demonstrated confounding because they both explained the same variability in the response variable. Males had higher aerobic fitness levels than females (r=-0.37, p=0.001) and gender was a stronger predictor of hematologic and PV changes at altitude than aerobic fitness level. Including fitness level as a predictor limited the data set to 271 subjects with only six females demonstrating a sea-level VO_{2max} ≥50 ml/kg/min. When females were eliminated from the analysis, fitness level was not a strong predictor of the hematologic and PV response to altitude and therefore not included in the final models.

Figure 1

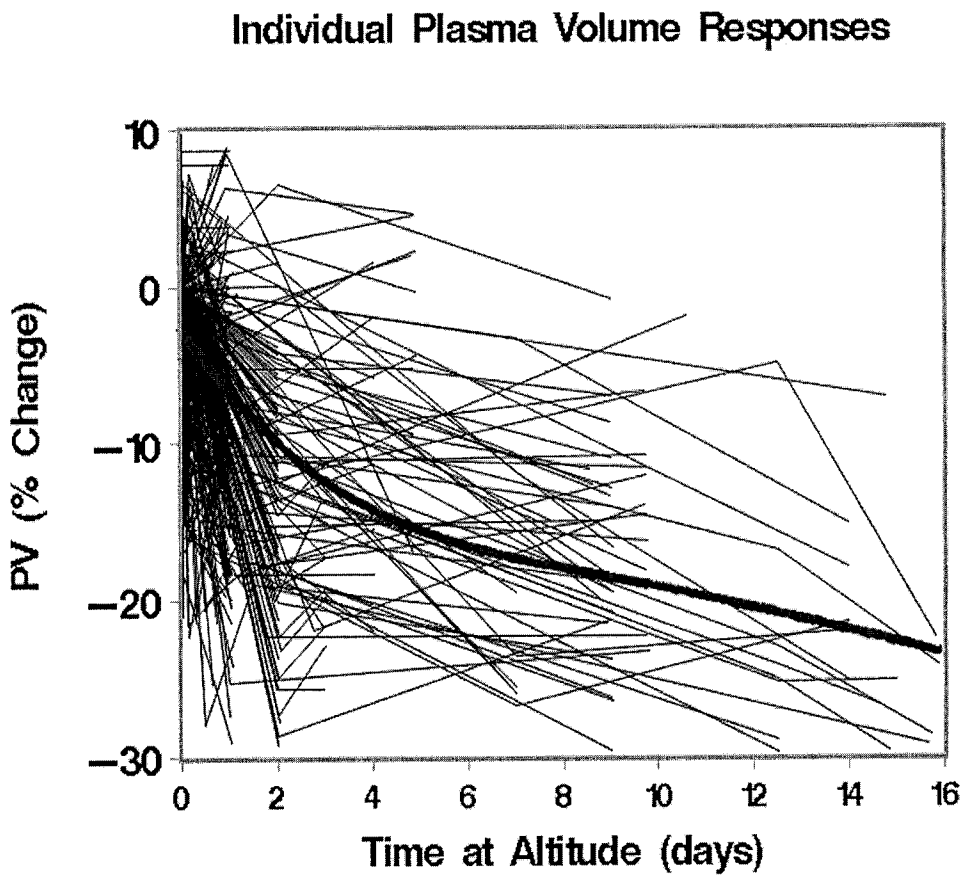

Figure 1. Individual profiles of the change in plasma volume (PV) over time at altitude for the 361 subjects included in this data set. The average trend line demonstrates a rapid drop in PV that eventually slows down and plateaus as the altitude exposure duration continues. Exploratory analysis indicates that a linear and quadratic effect of time should be included in the model.

Figure 2

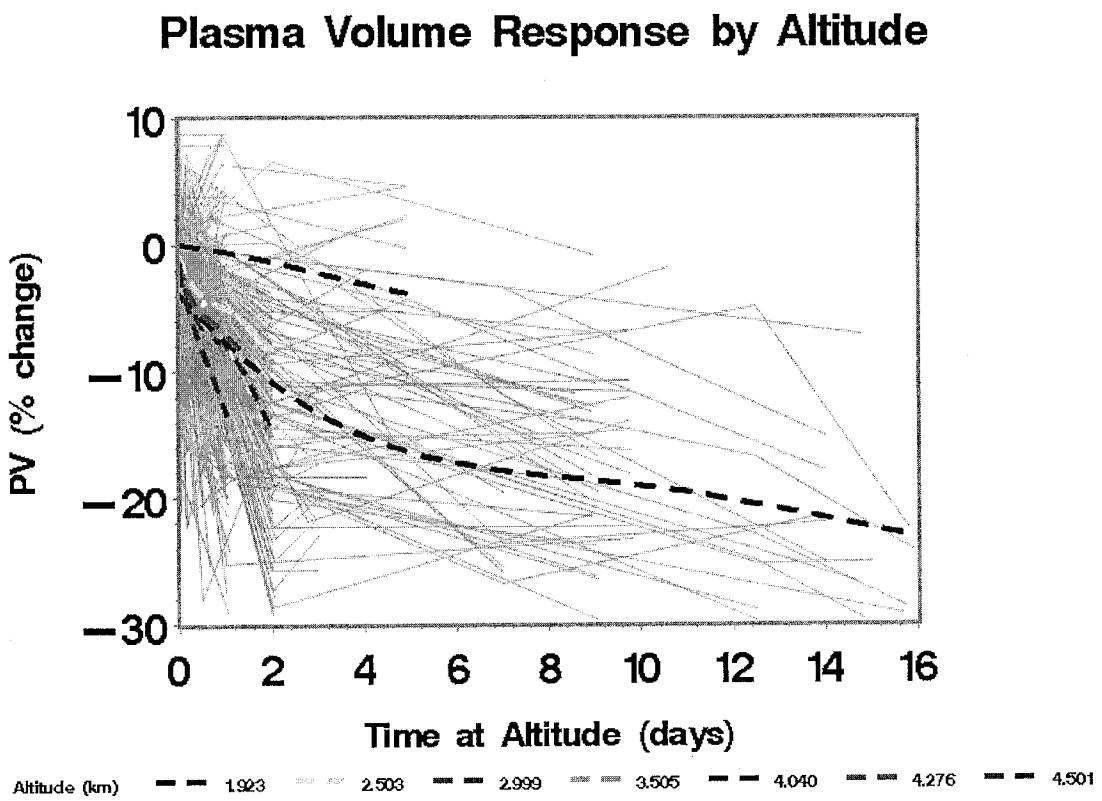

Figure 2. Individual profiles of the change in plasma volume (PV) over time at various altitudes for the 361 subjects in the data set. The average trend line through the data indicates that altitude would be a likely candidate to include in the model. Exploratory data analysis was performed for all demographic variables such as gender, age, height, weight, fitness level, race, smoking status, and others to determine likely candidates for all three models.

Table 2 presents the final parameter estimates and equations of fitting several multilevel models for change to the hematologic and PV data collected over time at various altitudes. The three equations for [Hb], Hct, and PV over time at altitude are slightly different but all include time at altitude, the quadratic effect of time at altitude, altitude, gender, initial hematologic status, and interactions among these variables as significant predictors in the models. Initial hematologic status is not included in the PV equation given that the change in PV is assumed to be zero at SL and the change at altitude is based on initial sea-level values of [Hb] and Hct. The pseudo R^2 statistic assesses the proportion of total outcome variation explained by the individual growth

model's specific combination of predictors. The final predictors in the [Hb], Hct, and PV models explained 91%, 90% and 77%, respectively, of the total outcome variation.

Table 2. Equations using parameter estimates to predict the change in hemoglobin concentration ([Hb]), hematocrit (Hct), and plasma volume (PV) from initial sea-level values following rapid ascent to various altitudes in unacclimatized men and women.

INPUTS	Exposure Time: Time (days) Altitude: ALT (km) Gender: (male=0, female=1) SLHb; Initial Sea-Level Hemoglobin Concentration SLHct: Initial Sea-level Hematocrit
TRANFORMATIONS/ CONSTANTS	Centered Hemoglobin (CHb): SLHb-14.2 Centered Hematocrit (CHct): SLHct-42.2
Hemoglobin Concentration [Hb] (g/dl)	[Hb] = 14.203 + (Time*0.2155) - (Time^2*0.0112) + (ALT*0.1635) + (ALT*Time*0.0181) - (Gender*.0002) -(Gender*Time*0.1481) – (Gender*ALT*0.1194) + (CHb*0.9987) - (CHb*Time*.1365) + (CHb*Time^2*0.0119) - (CHb*ALT*0.0498)
Hematocrit (Hct) (%)	Hct = 42.21 + (Time*0.4906) - (Time^2*0.0573) + (ALT*0.3870) + (ALT*Time*0.1550) - (Gender*.0220) -(Gender*Time*0.4790) – (Gender*ALT*0.3471) + (CHct*0.9976) - (CHct*Time*.1115) + (CHct*Time^2*0.0119) - (CHct*ALT*0.0663)
Plasma Volume (PV) Change (%)	PV = -0.1064 - (Time*3.3411) + (Time^2*0.1688) – (ALT*1.1954) + (Gender*0.2297) + (Gender*Time*0.8448)

[Hb]=Hemoglobin concentration; Hct=Hematocrit; PV=Plasma Volume; ALT=Altitude; CHb= Sea-level [Hb]-14.2; CHct=Sea-level Hct-42.2. The parameter estimates are per 24 h increase in TIME, 1000 m increase in ALT and the reference category for gender is male. The intercept represents the initial starting value (Time=0) for a male (Gender=0) at sea level (ALT=0 m). Use of sample equations for calculation of [Hb], Hct, and PV change at altitude are subject to patents pending

Figure 3 represents the population average change in [Hb] following rapid ascent to various altitudes ranging from 2000-4500 m in unacclimatized men and women. Both the linear ($P<0.0001$) and quadratic ($P<0.0001$) effects of time were significant predictors in the [Hb] model. Both genders demonstrated a dramatic increase in [Hb] after one day of altitude exposure (~0.4 g/dl) which eventually slowed down with continued altitude exposure (Tables 3-4). Gender, however, impacted the pattern of increase in [Hb] over time at altitude ($P<0.0001$). Men continued to increase their [Hb] by 0.3 g/dl for every 2 days of altitude exposure while women only increased [Hb] by 0.1 g/dl for every 2 days of altitude exposure. The increase in [Hb] plateaued following 6 days of altitude exposure in women while [Hb] did not level off in men until the 12^{th} day of exposure. Altitude ($P<0.0001$) was also a significant factor in the [Hb] model. For every 500 m increase in altitude from SL, [Hb] increases by ~ 0.1 g/dl regardless of gender or duration of exposure (Tables 3-4). The rate of increase in [Hb] was also dependent on initial levels of [Hb] ($P<0.0001$). Individuals with a low initial [Hb] demonstrated a more dramatic increase over time at a given altitude than those with a high initial [Hb]. Exact estimates taking into account the effect of initial starting levels of [Hb] over time at altitude can be calculated utilizing the equations contained in Table 2.

Figure 3

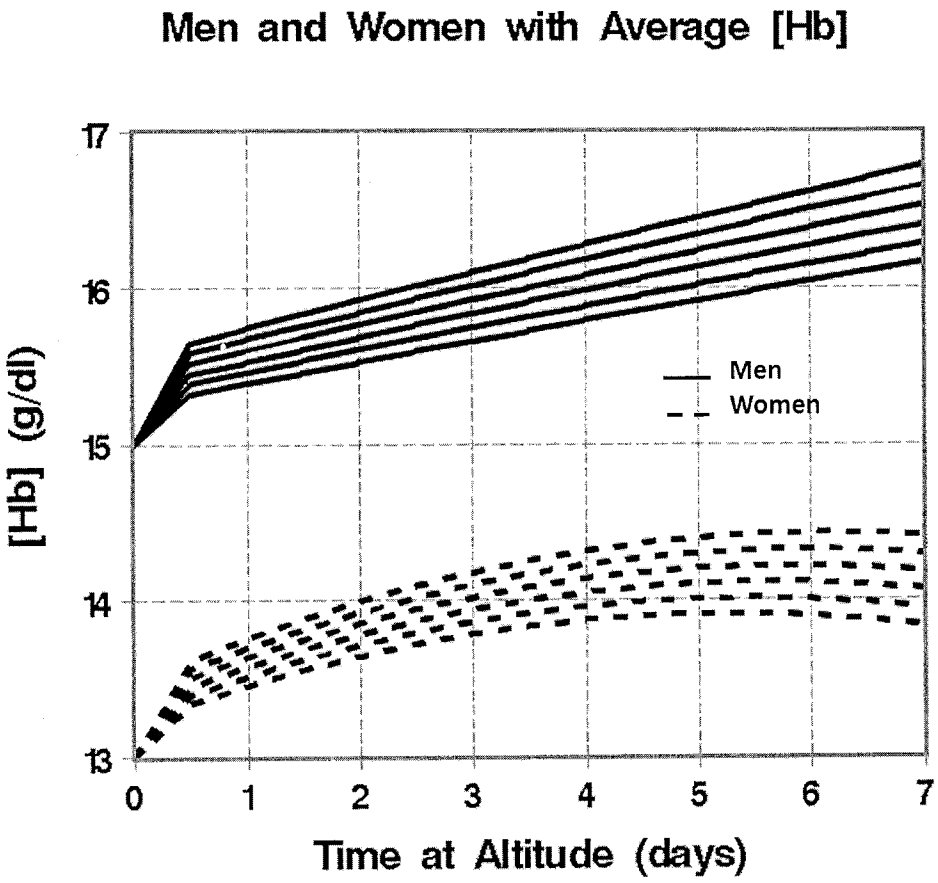

Figure 3. This figure demonstrates predictions of hemoglobin ([Hb]) over the first 7 days of altitude exposure in men and women after rapid ascent to altitudes ranging from 2000-4500 m starting at the mean initial sea level [Hb] in both men and women. The lowest group of lines starts at 2000 m and increases by 500 m until reaching 4500 m for the top group of lines

Table 3. Estimates of the increases in hemoglobin concentration ([Hb]) and hematocrit (Hct) following 7 days of exposure to various altitudes in men starting with a sea-level [Hb] of 15 g/dl and Hct of 44%.

Altitude (m)	Days at Altitude							
	1		3		5		7	
	[Hb] (g/dl)	Hct (%)	[Hb] (g/dl)	Hct (%)	[Hb] (g/dl)	Hct (%)	[Hb] (g/dl)	Hct (%)
2000	15.4±0.1	45.1±0.3	15.7±0.3	46.0±1.3	15.9±0.5	46.5±1.3	16.2±0.6	46.7±1.9
2500	15.5±0.1	45.3±0.2	15.8±0.2	46.3±0.6	16.0±0.4	47.0±1.0	16.3±0.5	47.4±1.4
3000	15.5±0.1	45.5±0.2	15.8±0.2	46.7±0.5	16.1±0.3	47.5±0.7	16.4±0.3	48.0±1.0
3500	15.6±0.1	45.7±0.2	15.9±0.2	47.1±0.4	16.2±0.2	48.1±0.6	16.5±0.2	48.7±0.7
4000	15.7±0.1	45.9±0.2	16.0±0.2	47.4±0.4	16.3±0.2	48.6±0.6	16.7±0.2	49.4±0.6
4500	15.8±0.1	46.2±0.3	16.1±0.2	47.8±0.6	16.4±0.3	49.1±0.8	16.8±0.3	50.1±1.0

Table 4. Estimates of the increases in hemoglobin concentration ([Hb]) and hematocrit (Hct) following 7 days of exposure to various altitudes in women starting with a sea-level [Hb] of 13 g/dl and Hct of 39%.

Altitude (m)	Days at Altitude							
	1		3		5		7	
	[Hb] (g/dl)	Hct (%)	[Hb] (g/dl)	Hct (%)	[Hb] (g/dl)	Hct (%)	[Hb] (g/dl)	Hct (%)
2000	13.4±0.1	40.1±0.4	13.8±0.4	40.8±1.6	13.9±0.6	40.8±1.6	13.8±0.8	40.1±2.2
2500	13.5±0.1	40.3±0.3	13.9±0.3	41.1±0.8	14.0±0.5	41.3±1.3	14.0±0.6	40.8±1.8
3000	13.6±0.1	40.5±0.3	13.9±0.2	41.5±0.7	14.1±0.4	41.8±1.0	14.1±0.5	41.4±1.4
3500	13.6±0.1	40.7±0.3	14.0±0.2	41.8±0.6	14.2±0.3	42.3±0.9	14.2±0.4	42.1±1.1
4000	13.7±0.1	40.9±0.3	14.1±0.2	42.2±0.6	14.3±0.3	42.8±0.8	14.3±0.4	42.8±1.0
4500	13.8±0.1	41.1±0.3	14.2±0.2	42.6±0.6	14.4±0.3	43.3±0.9	14.4±0.4	43.4±1.2

Figure 4 represents the population average change in Hct following rapid ascent to various altitudes ranging from 2000-4500 m in unacclimatized men and women. Both genders demonstrated a dramatic increase in Hct after one day of altitude exposure (~1.1%) which eventually slowed down with continued altitude exposure (Tables 3-4). For every 500 m increase in altitude after the first 24 h of exposure, Hct

was increased by 0.2-0.6% depending on the altitude. Gender, however, impacted the pattern of increase in Hct over time at altitude ($P<0.0001$). Men increased their Hct by ~0.9 to1.6%, ~0.5 to 1.3% and ~0.2 to 1.0% for every 2 days of altitude exposure after the first 24 h of exposure with larger increases at higher altitudes. Women, on the other hand, increased their Hct by ~0.7 to 1.5%, ~0.0 to 0.7% and ~-0.1 to 0.1% for every 2 days of altitude exposure after the first 24 h of exposure with larger increases at the higher altitudes. The increase in Hct plateaued following 5 days of exposure in women and 9 days of exposure in men. In addition, for a given duration of exposure, those with a low initial Hct are impacted to a greater degree by an increase in altitude compared to those with a high initial Hct ($P<0.0001$). Exact estimates of the impact of initial starting levels of Hct on the altitude response can be calculated from the equations provided in Table 2.

Figure 4

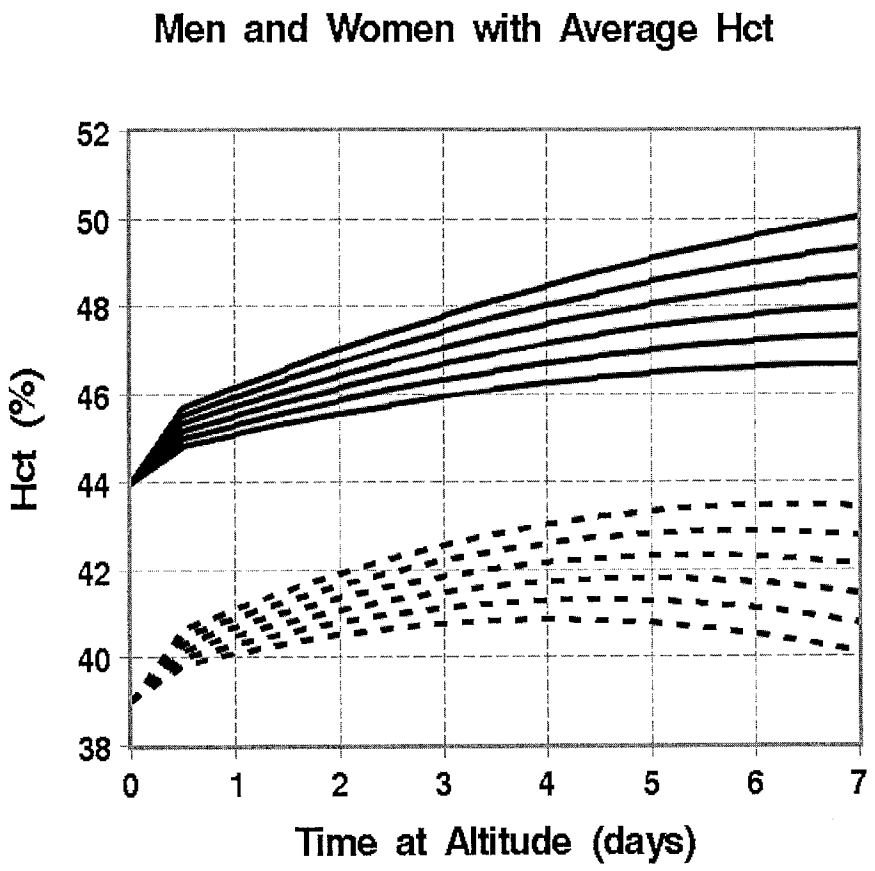

Figure 4. This figure demonstrates predictions of hematocrit (Hct) over the first 7 days of altitude exposure in men and women after rapid ascent to altitudes ranging from 2000-4500 m starting at the mean initial Hct at sea level in both men and women. The lowest group of lines starts at 2000 m and increases by 500 m until reaching 4500 m for the top group of lines

Figure 5 represents the PV change following rapid ascent to various altitudes ranging from 2000-4500 m in unacclimatized men and women. The drop in PV occurs rapidly within the first 24 h of exposure and then levels off with increasing exposure duration. Table 4 contains estimates of the PV decrease at various altitudes in men and women. Both men and women demonstrate a ~ 5% decline in PV after the first day at 2000 m altitude. With every 500 m increase in altitude from 2000 m, there is an additional 0.6% decrease in PV after the first day. For every 2 days of exposure after the first day of exposure, men demonstrate an additional 3-5% decrease in PV whereas women demonstrate an additional 1-3% decrease. The decline in PV over time at

altitude tends to level off in women after 7 days while men reach a plateau after 9 days. Altitude was a significant factor ($P<0.0001$) in predicting the decrement in PV with a 0.6% greater decrement with every 500 m increase in elevation from SL. Men have a greater initial drop in PV than women ($P<0.0001$) and demonstrate a steeper decline with increasing duration of altitude exposure for a given altitude.

Figure 5

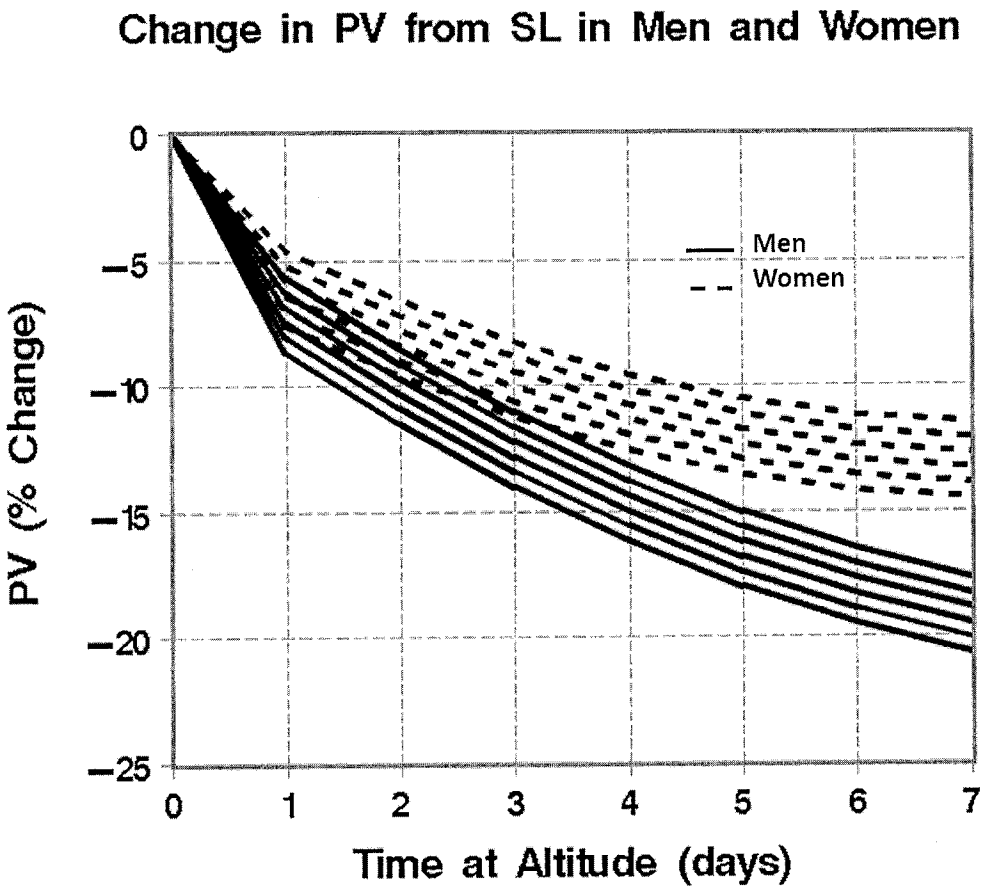

Figure 5. This figure demonstrates predictions of the change in plasma volume (PV) over the first 7 days of altitude exposure to 2000-4500 m in men and women after rapid ascent. The lowest group of lines starts at 2000 m and increases by 500 m until reaching 4500 m for the top group of lines

Table 4. Estimates of the change in Plasma Volume (%) following 7 days of exposure to various altitudes in men and women.

Altitude (m)	Days at Altitude							
	1		3		5		7	
	Men	Women	Men	Women	Men	Women	Men	Women
2000	-5.7±0.8	-4.6±1.0	-11.0±1.7	-8.2±2.1	-14.9±2.2	-10.5±2.8	-17.6±2.8	-11.5±3.3
2500	-6.3±0.8	-5.2±1.0	-11.6±1.7	-8.8±2.0	-15.6±2.2	-11.1±2.8	-18.2±2.3	-12.1±3.3
3000	-6.9±0.8	-5.8±1.0	-12.2±1.7	-9.4±2.0	-16.2±2.2	-11.7±2.8	-18.8±2.3	-12.6±3.3
3500	-7.5±0.8	-6.4±1.0	-12.8±1.6	-10.0±2.0	-16.8±2.1	-12.3±2.7	-19.4±2.3	-13.3±3.3
4000	-8.0±0.8	-7.0±1.0	-13.4±1.6	-10.6±2.0	-17.4±2.4	-12.9±2.7	-20.0±2.2	-13.9±3.2
4500	-8.6±0.8	-7.6±1.1	-14.0±1.6	-11.2±1.9	-18.0±2.1	-13.5±2.7	-20.6±2.2	-14.5±3.2

DISCUSSION

The US Anti-Doping Agency (USADA) requested that the US Army Research Institute of Environmental Medicine (USARIEM) answer the following questions using the USARIEM Mountain Medicine Database: 1) predict human hematologic and PV responses of unacclimatized lowlanders to rapid ascent and stay at moderate to high-altitudes, 2) quantify the changes in hemoglobin [Hb], hematocrit (Hct), and PV as a result of a given gain in terrestrial elevation and delineate the time course of these changes, 3) determine baseline demographics and physiologic descriptors that are important in predicting these changes and 4) establish the time course for altitude-induced changes in [Hb], Hct and PV to return to normal sea-level values following descent.

Our laboratory has developed the first know prediction equations of the hematologic and PV response following rapid ascent and stay over various altitudes in unacclimatized lowlanders. These equations were developed using a relational database containing ascent profiles as well as data collected by the same research team using similar procedures under well-controlled experimental conditions (i.e, controlled temperature and humidity, adequate hydration, no medication use). In addition, the modelling procedures utilized to develop these predictions are applicable to the longitudinal data collected and account for the fact that hematologic measurements from different experiments were made at different time points. These equations can be easily incorporated into an excel spreadsheet or smartphone

application for widespread use in the sports community.

The sports agencies monitoring the blood profile of elite athletes training at altitude also have a need quick correction factors that can be applied to detect "cheating". These models allow us to estimate these correction factors and the level of error associated with them. Tables 2-4 provide detailed estimates of the hematologic and PV response at various altitudes over time at altitude which can be summarized using the following correction factors in terms of altitude: 1) 0.1 g/dl increase in [Hb] for every 500 m increase in altitude regardless of gender, 2) 1.1% increase in Hct at 2000 m and an additional 2-6% increase for every 500 m increase in altitude depending on the altitude, and 3) 5% decrease in PV in the first day of exposure to 2000 m with an additional 0.6% decrease for every 500 m increase > 2000 m. In regards to the effect of time at altitude, the following correction factors can be utilized: 1) 0.2 g/dl increase in [Hb] for every 2 days of exposure after the first 24 h with increases levelling off after the 6^{th} day of exposure in women and 12^{th} day of exposure in men, 2) 1.0% increase in Hct for every 2 days of exposure after the first 24 h with increases levelling off after the 5^{th} day of exposure in women and 9^{th} day of exposure in men, and 3) 3-5% decreases in PV in men and 1-3% decreases in women for every 2 days of exposure after the first 24 h which levels off after the 7^{th} day of exposure in women and 10^{th} day of exposure in men.

The most significant predictive factor (i.e, factor with the highest standardized estimate) in the models for predicting the change in [Hb] and Hct at altitude was the initial SL values of [Hb] and Hct. The model predicts that final values of [Hb] and Hct will be higher after 7 days of altitude exposure in individuals starting with high initial SL values of these parameters. However, the model also predicts that individuals with low starting values of [Hb] and Hct have accelerated increases in their hematologic status as a result of altitude exposure. A recent meta-analysis also found that the extent of the erythropoietic response to altitude is dependent on the initial red cell volume [8] with attenuated increases in individuals with high initial red cell volumes. In a similar manner, other studies reported that moderate-altitude residents starting with a 6%-13% higher [Hb] than sea-level residents demonstrate an attenuated increase in [Hb] as a result of exposure to a higher altitude [27,45]. The rough correction factors only consider one initial starting level of [Hb] and Hct for both men and women. Therefore, if exact estimates are needed, the equations must be applied.

The second most important factor in predicting the change in [Hb] and Hct

following altitude exposure was the level of exposure. Given that the change in [Hb] and Hct during the acute response to altitude is driven by the loss in PV, the degree of hemoconcentration at altitude depends largely on the PV response to altitude [7]. The loss in PV that occurs at altitude is not due to dehydration, given that most individuals demonstrated normal urine osmolality, but is most likely mediated by protein loss due to increased capillary permeability to protein at altitude [23]. The resultant reduced vascular oncotic pressure at altitude likely causes less fluid to be held within the vascular space [23]. A review article reported a greater magnitude of decrease in PV in individuals exposed to higher elevations during short-term (4-16 days) stays at various altitudes [7]. The maximum decrease in PV in men as a result of altitude exposure from that review article was similar to the PV response predicted from our models at similar altitudes. Studies on long-term altitude residents also demonstrate higher levels of [Hb] and Hct in high-altitude (3100 m) compared to moderate-altitude (1600 m) residents [31] but this hemoconcentration is likely due to erythrocyte volume expansion as PV returns to normal levels following 4-8 months of altitude exposure [13].

Time was the third most important factor in predicting the changes in hematologic and PV status over varying altitudes. The models contained in this report for the first time characterize the time-varying nature of changes in [Hb], Hct, and PV with altitude exposure as well as the interaction between the duration of exposure and the level of exposure. The greatest decrease in PV occurs in the first 24 hours and then the rate of decrease declines as the altitude exposure increases. In addition, larger decreases in PV occurred at higher elevations. However, the pattern of decrease in PV was not impacted by altitude with each altitude demonstrating a similar daily decrement over the duration of exposure. Others have reported that the initial decrease in PV recovers following long-duration exposure to altitude [24,46] but the time-course of this recovery has never been defined. These models predict that the recovery of PV back to SL values while at altitude takes 7-10 days depending on gender.

Gender was also a significant factor when evaluating demographics that impacted the change in [Hb], Hct, and PV with altitude exposure. Although both men and women demonstrated a large increase in [Hb] and Hct within the first 24 h of exposure to various altitudes, the pattern of increase with continued altitude exposure was different between the two genders. Women tended to reach their maximal Hct and [Hb] values after 5-6 days of altitude exposure while men tended to reach maximal

levels following 9-12 days of exposure. This is the first time that a gender effect has been identified when examining hematologic and PV changes at altitude. The reasons for this difference are not readily understood. Women compared to men typically demonstrate lower total blood volumes due to both reduced PV and red cell volume [47]. Although this difference disappears when normalized to lean body mass [48], the recovery of PV after an initial loss with altitude exposure may take less time in women. In addition, hormonal factors that may contribute to differences in endothelial permeability may cause greater retention of water in women [49]. More work is needed to elucidate other potential physiologic mechanisms for the gender differences in the PV response to altitude exposure.

Fitness level was a significant factor ($P<0.001$) in the models and initially included in the analysis. However, when model assumptions were evaluated, fitness level was dropped as it was determined that confounding existed with gender and fitness level. The women had lower fitness levels than the men and the changes in [Hb], Hct, and PV were reflective of the gender difference rather than fitness level. When fitness level was examined only in males, fitness level dropped out as a significant predictor of changes in hematologic and PV status after rapid ascent to altitude. Although highly-trained athletes consistently demonstrate higher blood volumes, plasma volumes and red cell volumes than non-trained individuals, their [Hb] and Hct are similar due to equal increases in all three blood compartments [7,50-52]. In fact, in some studies, [Hb] and Hct are lower in athletes due to expanded plasma volumes in relation to red cell volumes [53]. Therefore, it was not surprising that fitness level dropped out when the effect of gender on fitness level was removed from the model.

Although age was eliminated from the final models, others have reported lower [Hb] and Hct with increasing age [54] as well as decreased hemoconcentration with aged individuals during fixed stay at a given altitude [20,21]. This is particularly prominent in males given that testosterone is a known stimulant of red blood cell production [55,56] and levels decrease substantially in men with aging [57]. Although we did not detect age as a factor in these models, most of our volunteers were relatively young. For the purpose of detecting "cheating" in sports, most individuals would be relatively young and the effects of age would not be an important predictor. Race was also not a significant factor in these models even though others have shown that Caucasians typically have increased [Hb] and Hct values compared to African

Americans [54,58]. In our data analysis, however, all races other than whites were included in the non-white category due to a limited number of non-whites exposed to the various altitudes. The effect of race on hematologic and plasma volume status with altitude exposure therefore remains unknown. Weight, height, and body-mass index category were also not significant factors in the models which supports previous research suggesting no effect when basic blood parameters are standardized by these variables [48]. Smoking typically induces an elevated [Hb] and Hct due to stimulation of the bone marrow as a result of reduced oxygen delivery to the tissues [59]. Given the low percentage of smokers in our data set, we did not detect this effect.

Although not included in the model, the hematologic and PV status following return to SL after a prolonged altitude exposure can be estimated from previous research (See Table 1). One of the hallmarks of successful altitude acclimatization involves an increase in [Hb] and Hct over the duration of altitude exposure [60]. The time required for these indices to return to SL values upon descent depends on the duration and elevation of exposure as well as the activity and training performed while at altitude. Table 1 provides a list of all the known studies done that have measured [Hb], Hct, and PV changes during deacclimatization to altitude. The general conclusions you can draw from the summary of these studies is that when exposures are below 4000 m, most studies find that hematologic status returns very quickly to pre-ascent values [14,32-34,36]. Only one study below 4000 m found a significantly elevated [Hb] on days 1 and 4 following return to SL but vigorous physical training was conducted in this study [35]. From 4000-5000 m, the results are somewhat mixed. A few studies have reported significant increases in [Hb] and Hct for at least a week following return to SL [13,15] while others demonstrate no increase after 1-2 weeks at SL [16,17,19]. On the other hand, almost all of the mountain climbing expeditions to extreme altitudes report that [Hb] and Hct remained elevated for 17-30 days following expeditions lasting 40-130 days [12,18,37,38]. In summary, following exposure to altitudes below 4000 m, PV quickly recovers to pre-ascent values following return to SL and likely recovers within 2 weeks at altitudes above 4000 m. When individuals are exposed to extreme altitudes for long durations, the recovery may take 1-2 months or longer.

The limitations of this study should be acknowledged. First, the age range is limited to 18-45 yr. Therefore, age effects on hematologic and plasma volume status outside of these ranges cannot be determined. Second, the number of non-whites and

smokers in the database was limited and may have contributed to the lack of significance for race or smoking status in the models. Third, differing blood auto-analyzers and methods were used to measure [Hb] and Hct. Given that all measurements were made in the same laboratory using competent skilled technicians with many years of experience, the variability introduced can be accounted for by the models. The models explain 77-91% of the variability in the outcome measures which increases our confidence that these models predict with a high degree of accuracy despite the limitations. Fourth, PV was not measured but calculated based on [Hb] and Hct values. Although, errors can be associated with this calculation, in euhydrated controlled conditions, the estimates of changes in PV are consistent across studies. Last, the results are limited to the first 7 days at altitude.

CONCLUSIONS

We developed prediction models from a relational database containing hematological variables collected over time at various altitudes using standardized procedures. Our models quantify changes in hematologic and PV status over a wide range of altitudes and time points. The results suggest that initial SL hematologic status, level of altitude exposure, duration of exposure, initial starting levels of [Hb] and Hct, and gender all effect the increase in [Hb] and Hct with rapid ascent and stay at fixed altitudes. The increase in [Hb] and Hct for a given duration of altitude exposure occurs rapidly in the first 24-h of exposure due to a loss of PV and begins to plateau with increasing duration of exposure. The plateau is gender dependent with hematologic status in women levelling off in 5-6 days while men take 9-12 days of altitude exposure. The extent of increase in [Hb] and Hct is dependent on the level of exposure with greater decreases in PV occurring at higher altitudes. In addition, regardless of gender, those individuals with high starting values of [Hb] and Hct show less of an increase with altitude exposure compared to those individuals with lowest starting values. Fitness level, age, height, weight, race, body-mass index, and smoking status were not significant predictors of the hematologic and PV status at altitude.

The overall impact of altitude on hematologic and PV status in unacclimatized lowlanders can be summarized using the following correction factors after one day at altitude: 1) 0.1 g/dl increase in [Hb] for every 500 m increase in altitude regardless of gender, 2)1.1% increase in Hct at 2000 m and an additional 2-6% increase for every

500 m increase in altitude depending on the altitude, and 3) rapid 5% loss in PV at 2000 m and additional 0.6% decrease with every 500 m above 2000 m. For the first time, governing bodies can apply a science-based correction factor developed from a comprehensive data set to account for the effect of altitude on hematologic status when athletes engage in short-term training at altitude. These models will greatly enhance the ability of the US Anti-Doping Agency and other governing bodies to detect "unfair practices" in athletes seeking to gain an edge in their performance through illegal manipulation of their erythrocyte volume.

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